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53. The plant cell of claim 52, wherein said fourth coding sequence comprises at least a portion of the nucleotide sequence set forth in SEQ ID NO: 24.

5 54. The plant cell of claim 52 further comprising in its genome a fifth DNA construct comprising a promoter that drives expression in a plant cell operably linked to a fifth coding sequence, wherein said fifth coding sequence encodes an NADH kinase or an NAD⁺ kinase and said fifth coding sequence is operably linked to a nucleotide sequence encoding a peroxisome-targeting signal.

10

55. The plant cell of claim 54, wherein said fifth coding sequence comprises at least a portion of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25-27.

15 56. A plant cell genetically manipulated to produce polyhydroxyalkanoate in its peroxisomes, said plant cell comprising in its genome:

a stably integrated first DNA construct comprising a promoter that drives expression in a plant cell operably linked to a first coding sequence, wherein said first coding sequence encodes a polyhydroxyalkanoate synthase and is operably
20 linked to a nucleotide sequence encoding a peroxisome-targeting signal;

a stably integrated second DNA construct comprising a promoter that drives expression in a plant cell operably linked to a second coding sequence, wherein said second coding sequence encodes an acetyl-CoA:acetyl transferase and is operably linked to a nucleotide sequence encoding a peroxisome-targeting signal; and

25 a stably integrated third DNA construct comprising a promoter that drives expression in a plant cell operably linked to a third coding sequence, wherein said third coding sequence is operably linked to a nucleotide sequence encoding a peroxisome-targeting signal and said third coding sequence is selected from the group consisting of:

- 30 (a) a nucleotide sequence encoding a 3-ketoacyl-CoA reductase that is capable of utilizing NADH;
- (b) a nucleotide sequence set forth in SEQ ID NO: 3;
- (c) a nucleotide sequence set forth in SEQ ID NO: 22;

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- (d) the nucleotide sequence set forth in SEQ ID NO: 1;
- (e) a nucleotide sequence encoding a multifunctional protein-2, wherein the hydratase activity of said multifunctional protein has been eliminated; and
- 5 (f) the nucleotide sequence set forth in SEQ ID NO: 6.

57. The plant cell of claim 56, wherein said second coding sequence comprises at least a portion of the nucleotide sequence set forth in SEQ ID NO: 24.

10 58. A plant cell genetically manipulated for the synthesis in its peroxisomes of at least one intermediate molecule in polyhydroxyalkanoate synthesis, said plant cell comprising in its genome at least one stably incorporated DNA construct comprising a coding sequence for an enzyme involved in the synthesis of said intermediate molecule, said coding sequence operably linked to a promoter that
15 drives expression in a plant cell and to a nucleotide sequence encoding a peroxisome-targeting signal, wherein said coding sequence is selected from the group consisting of:

- (a) a nucleotide sequence encoding a 2-enoyl-CoA hydratase that is capable of catalyzing the synthesis of
20 R-(-)-3-hydroxyacyl-CoA ;
- (b) a nucleotide sequence set forth in SEQ ID NO: 21;
- (c) a nucleotide sequence comprising the 2-enoyl-CoA hydratase domain of a multifunctional protein-2.
- (d) a nucleotide sequence set forth in SEQ ID NO: 4;
- 25 (e) a nucleotide sequence set forth in SEQ ID NO: 1;
- (f) a nucleotide sequence encoding a multifunctional protein-2, wherein the dehydrogenase activity of said multifunctional protein has been eliminated; and
- (g) a nucleotide sequence encoding a 3-ketoacyl-CoA reductase that is capable of utilizing NADH;
- 30 (h) a nucleotide sequence set forth in SEQ ID NO: 3;
- (i) a nucleotide sequence set forth in SEQ ID NO: 22;
- (j) the nucleotide sequence set forth in SEQ ID NO: 1;

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- (k) a nucleotide sequence encoding a multifunctional protein-2, wherein the hydratase activity of said multifunctional protein has been eliminated; and
- (l) the nucleotide sequence set forth in SEQ ID NO: 6.

5

59. The plant cell of claim 58, wherein said intermediate molecule is an R-(-)-3-hydroxyacyl-CoA or a 3-ketoacyl-CoA.

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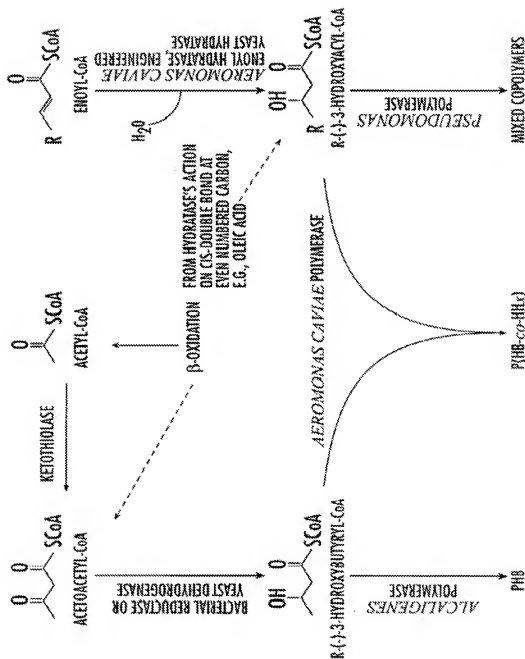


FIG. 1.

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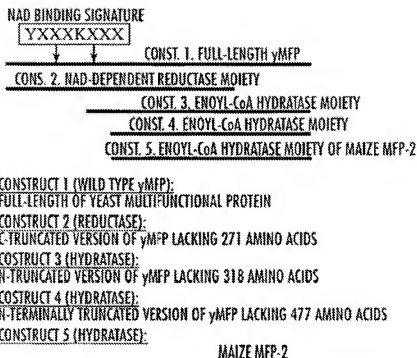


FIG. 2

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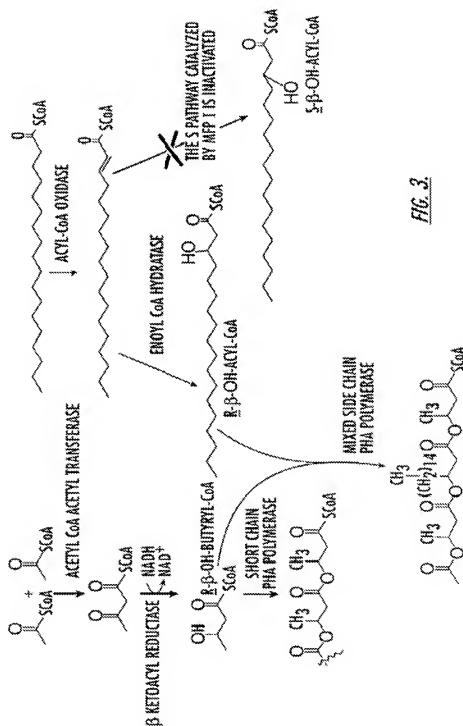


FIG. 3.

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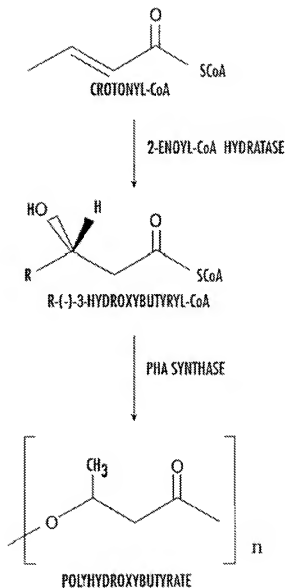


FIG. 4.

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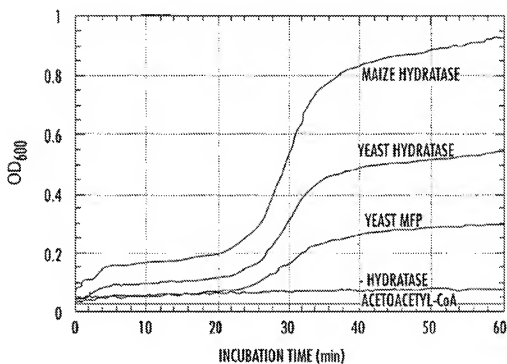


FIG. 5

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 Li, Chun Ping
 Gong, Jian G
 Hitz, William D
 Liebergesell, Matthias
 Dhugga, Kanwarpal S
 Briggs, Kristen K

<120> PRODUCTION OF POLYHYDROXYALKANOATE IN PLANTS

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Leu Gly Phe Val Asp Val Pro Gly Leu Asn Phe Asp Ala Ser Leu Leu	
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Leu His Gly Gln Gln Tyr Ile Glu Ile Tyr Arg Pro Ile Pro Ser Tyr	
100 105 110	
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35 40 45
Asp Glu Lys Glu Leu His Phe Val Tyr His Arg Asp Gly Gln Pro His
50 55 60
Ile Lys Thr Leu Pro Thr Phe Val Ser Leu Phe Pro Asn Lys Asn Ser
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Asn Gly Leu Gly Phe Val Asp Val Pro Gly Leu Asn Phe Asp Ala Ser
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165 170 175
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180 185 190
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195 200 205
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Gln Arg Val Asp Ile Leu Val Asn Asn Ala Gly Ile Leu Arg Asp Lys
20 25 30
tct ttt tta aaa atg aaa gat gag gaa tgg ctt gct gtc ctg aaa gtc 144
Ser Phe Leu Lys Met Lys Asp Glu Trp Phe Ala Val Leu Lys Val
35 40 45
cac ctt ttt tcc aca ttt tca ttg tca aaa gca gta tgg cca ata ttt 192
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Thr Lys Gln Lys Ser Gly Phe Ile Ile Asn Thr Thr Ser Thr Ser Gly
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85 90 95

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Thr Leu Ala Lys Ala Val Lys Phe Pro Thr Pro Ile Leu His Gly Leu	
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tgt acc tta ggt att agt gcg aaa gca ttg ttt gaa cat tat ggt cca	1392
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<223> Nucleotides 4-1566 of SEQ ID NO: 4 corresponds to nucleotides 1381-2943 of SEQ ID NO: 3.

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Ser Phe Leu Lys Met Lys Asp Glu Glu Trp Phe Ala Val Leu Lys Val
35 40 45

His Leu Phe Ser Thr Phe Ser Leu Ser Lys Ala Val Trp Pro Ile Phe
50 55 60

Thr Lys Gln Lys Ser Gly Phe Ile Ile Asn Thr Thr Ser Thr Ser Gly
65 70 75 80

Ile Tyr Gly Asn Phe Gly Gln Ala Asn Tyr Ala Ala Lys Ala Ala
85 90 95

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130 135 140

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Ser Gly Arg Arg Val Ile Gly Gln Leu Phe Glu Val Gly Gly Gly Trp
165 170 175

Cys Gly Gln Thr Arg Trp Gln Arg Ser Ser Gly Tyr Val Ser Ile Lys
180 185 190

Glu Thr Ile Glu Pro Glu Glu Ile Lys Glu Asn Trp Asn His Ile Thr
195 200 205

Asp Phe Ser Arg Asn Thr Ile Asn Pro Ser Ser Thr Glu Glu Ser Ser
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Met Ala Thr Leu Gln Ala Val Gln Lys Ala His Ser Ser Lys Glu Leu
225 230 235 240

Asp Asp Gly Leu Phe Lys Tyr Thr Thr Lys Asp Cys Ile Leu Tyr Asn
245 250 255

Leu Gly Leu Gly Cys Thr Ser Lys Glu Leu Lys Tyr Thr Tyr Glu Asn
260 265 270

Asp Pro Asp Phe Gln Val Leu Pro Thr Phe Ala Val Ile Pro Phe Met
275 280 285

Gln Ala Thr Ala Thr Leu Ala Met Asp Asn Leu Val Asp Asn Phe Asn
290 295 300

Tyr Ala Met Leu Leu His Gly Glu Gln Tyr Phe Lys Leu Cys Thr Pro
305 310 315 320

Thr Met Pro Ser Asn Gly Thr Leu Lys Thr Leu Ala Lys Pro Leu Gln
325 330 335

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Val Leu Asp Lys Asn Gly Lys Ala Ala Leu Val Val Gly Gly Phe Glu
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Thr Tyr Asp Ile Lys Thr Lys Lys Leu Ile Ala Tyr Asn Glu Gly Ser
 355 360 365

Phe Phe Ile Arg Gly Ala His Val Pro Pro Glu Lys Glu Val Arg Asp
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Gly Lys Arg Ala Lys Phe Ala Val Gln Asn Phe Glu Val Pro His Gly
 385 390 395 400

Lys Val Pro Asp Phe Glu Ala Glu Ile Ser Thr Asn Lys Asp Gln Ala
 405 410 415

Ala Leu Tyr Arg Leu Ser Gly Asp Phe Asn Pro Leu His Ile Asp Pro
 420 425 430

Thr Leu Ala Lys Ala Val Lys Phe Pro Thr Pro Ile Leu His Gly Leu
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Cys Thr Leu Gly Ile Ser Ala Lys Ala Leu Phe Glu His Tyr Gly Pro
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Tyr Glu Glu Leu Lys Val Arg Phe Thr Asn Val Val Phe Pro Gly Asp
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Thr Leu Lys Val Lys Ala Trp Lys Gln Gly Ser Val Val Val Phe Gln
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 to nucleotides 241- 2127 of SEQ ID NO: 3.

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gct gga ggg gcc tta ggt aag gta tat gca cta gct tac gca agc aga 96
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 20 25 30

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Cys Asn Lys Val Val Val Thr Gly Ala Gly Gly Gly Leu Gly Lys	
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Trp Gln Arg Ser Ser Gly Tyr Val Ser Ile Lys Glu Thr Ile Glu Pro
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580 585 590

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595 600 605

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to nucleotides 241- 2127 of SEQ ID NO: 3.

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Gly His Asn Ser Lys Ala Ala Asp Leu Val Val Asp Glu Ile Lys Lys
50 55 60

Ala Gly Gly Ile Ala Val Ala Asn Tyr Asp Ser Val Asn Glu Asn Gly
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Leu Ile Asn Asn Ala Gly Ile Leu Arg Asp Val Ser Phe Ala Lys Met

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450 455 460

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Lys Thr Ile Ala Leu Glu Gly Ala Lys Arg Gly Ile Ile Val Asn Val
485 490 495

Ile Ala Pro His Ala Glu Thr Ala Met Thr Lys Thr Ile Phe Ser Glu
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515 520 525

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Trp Gln Arg Ser Ser Gly Tyr Val Ser Ile Lys Glu Thr Ile Glu Pro
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Glu Glu Ile Lys Glu Asn Trp Asn His Ile Thr Asp Phe Ser Arg Asn
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Thr Ile Asn Pro Ser Ser Thr Glu Glu Ser Ser Met Ala Thr Leu Gln
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<211> 328

<212> DNA

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<211> 1462

<212> DNA

<213> *Raphanus sativus*

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<211> 3728
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<213> *Saccharomyces cerevisiae*

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